APPENDIX G: THERMAL CYCLER 9700 TEMPERATURE CALIBRATION, TEMPERATURE NON-UNIFORMITY, AND SYSTEM PERFORMANCE DIAGNOSTIC TESTS

1 DNA THERMAL CYCLER 9700 TEMPERATURE CALIBRATION VERIFICATION TEST

1.1 Verifying the Temperature Calibration

Perform the procedure as described in the following steps.

1.1.1 If the sample block heated cover is in the forward position, lift the lever, then slide the heated cover back.

1.1.2 Place the 9700 Temperature Verification Frame on the sample block.

1.1.3 Coat well A6 with mineral oil using a cotton swab.

1.1.4 Place the 0.2 mL probe assembly into well A6. Carefully thread the probe wire through the channel in the 9700 temperature verification frame to prevent damage to the probe and lead wires.

1.1.5 Make sure the probe is connected to the digital thermometer and is seated properly. Close the cover carefully by sliding the heated cover forward, and pulling the lever down.

1.1.6 Turn on the digital thermometer and follow the instructions in the Temperature Verification Kit on how to operate the digital thermometer, Model 4500.

1.1.7 Turn on the GeneAmp PCR System 9700. The main menu appears:

1.1.7.1 Select Utilities (F4)

1.1.7.2 Select Diagnostics (F1)

1.1.7.3 Select TmpVer (F3)

1.1.8 The Temperature Verification screen appears, select Temp (F1). This automatically configures the system 9700 for the Calibration Verification Test. The following screen appears:

```
                Calibration Verification
Block temp = xx.xºC  Cover temp = xxxºC

Place probe in well A6

Run                                    Cancel
```

F1 F2 F3 F4 F5

1.1.9 Use the digital thermometer to take temperature readings of the sample well connected to the 0.2 mL Probe Assembly. You will take a reading at two different setpoint temperatures.

1.1.10 Select Run (F1) to start the Calibration Verification Test. The following screen appears with the setpoint values displayed.

```
Calibration Verification
Block temp = xx.xºC  Cover temp = xxxºC

Setpoint is 85ºC
Cover must be within 10ºC of 85ºC
                                    Cancel

F1        F2        F3        F4        F5
```

* The cover must be within 1ºC of 105ºC. It may take several minutes for the system to ramp up.

1.1.11 The Calibration Verification screen counts down the time until the setpoint is reached. When the "Stabilization at setpoint" value decrements to zero, read the digital thermometer.

1.1.12 Using the numeric keys, type the value displayed on the digital thermometer in the "Enter actual block temperature" field displayed on the screen.

* The digital thermometer displays a four-digit value; round this off to three digits before typing it in the Calibration Verification screen.

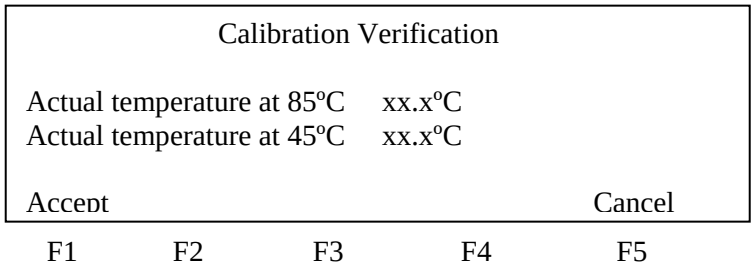
1.1.13 Press Enter on the keypad. The system automatically begins the second reading (45ºC setpoint). The Calibration Verification screen appears with the setpoint value displayed.

```
Calibration Verification
Block temp = xx.xºC  Cover temp = xxxºC

Setpoint is 45ºC
Cover must be within 30ºC of 45ºC
                                    Cancel

F1        F2        F3        F4        F5
```

* The cover must be within 1ºC of 105ºC.

1.1.14 Repeat steps 1.1.11 and 1.1.12, then press Enter from the keypad.

1.1.15 The system 9700 evaluates the calibration of the sample block temperature for the setpoint values that were entered and displays the results. A summary screen appears at the conclusion of the test.



1.1.16 Record the results on the Calibration Verification Test Data Sheet (Appendix J), then select Accept (F1).

1.1.17 If the sample block module is properly calibrated the Calibration Verification screen will display “Calibration is Good”

1.1.18 If the sample block module does not pass the Calibration Verification Test the screen will display “Instrument may require service. Contact Applied Biosystems Technical Support”

1.1.18.1 If the test fails, repeat the procedure to make sure the digital thermometer was not misread or that errors were not made entering data.

1.1.18.2 If the test fails again, contact Applied Biosystems Technical Support.

1.1.19 End the test by selecting Exit (F5).

2 TEMPERATURE NON-UNIFORMITY TEST

2.1 Verify the temperature uniformity of the system 9700 with a 0.2 mL sample block module

Perform the procedure as described in the following steps.

2.1.1 If the sample block heated cover is in the forward position, lift the lever, then slide the heated cover back.

2.1.2 Place the 9700 Temperature Verification Frame on the sample block.

2.1.3 Coat wells A1, A12, C4, C9, F4, F9, H1, and H12 with mineral oil using a cotton swab.

2.1.4 Place the 0.2mL probe assembly into well A1. Carefully thread the probe wire through the channel in the 9700 temperature verification frame to prevent damage to the probe and lead wires.

2.1.5 Make sure the probe is connected to the digital thermometer and is seated properly. Close the cover carefully by sliding the heated cover forward, and pulling the lever down.

2.1.6 Turn on the digital thermometer and follow the instructions in the Temperature Verification Kit on how to operate the digital thermometer, Model 4500.

2.1.7 Turn on the GeneAmp PCR System 9700. The main menu appears:

2.1.7.1 Select Utilities (F4)

2.1.7.2 Select Diagnostics (F1)

2.1.7.3 Select TmpVer (F3)

2.1.8 The Temperature Verification screen appears, select TNU (F2). This automatically configures the system 9700 for the Temperature Non-Uniformity Test. The following screen appears:

TNU Performance
Sample temp = xx.xºC Cover temp = xxxºC

Setpoint is 35ºC
Cover must be within 1ºC of setpoint
Cancel

F1 F2 F3 F4 F5

* It will take approximately 40 minutes for the cover temperature to reach the setpoint.

2.1.9 When the cover setpoint has been reached the following screen will appear:

```
                TNU Performance
Sample temp = xx.xºC  Cover temp = xxxºC

Place Probe in well A1
Press Run
Run                                  Cancel
```

F1 F2 F3 F4 F5

2.1.10 Press Run (F1) and the following screen appears with the setpoint value displayed.

```
                TNU Performance
Sample temp = xx.xºC  Cover temp = xxxºC

Setpoint is 37ºC
Sample must be within 1ºC of setpoint
                                     Cancel
```

F1 F2 F3 F4 F5

* The sample block must be within 1.0ºC of the setpoint and the cover must be within 1ºC of 35ºC. It may take a few minutes for the system to reach the setpoint temperature.

2.1.11 The TNU Performance screen counts down the time until the setpoint is stabilized. When the "Stabilization at setpoint" value decrements to zero, read the digital thermometer.

2.1.12 Using the numeric keys, type the value displayed on the digital thermometer in the "Enter actual block temperature" field displayed on the screen.

* The digital thermometer displays a four-digit value; round this off to three digits before typing it in the Calibration Verification screen.

2.1.13 Press enter on the keypad. The TNU Performance screen appears with setpoint value displayed at 94ºC. When the sample temp reaches 94ºC the setpoint value changes to 37ºC and the following screen appears.

```
                TNU Performance
Sample temp = xx.xºC  Cover temp = xxxºC

Place probe in well  A12
Press Run
Run                                  Cancel
```

F1 F2 F3 F4 F5

* During the 94ºC setpoint test this will be reversed. The first setpoint value displayed is 37ºC, when this sample temp setpoint is reached the setpoint value changes to 94ºC and the TNU Performance screen appears.

2.1.13 Slide the heated cover back and place the probe in A12. Make sure the probe is seated properly. Close the cover carefully by sliding the heated cover forward, and pulling the lever down. Repeat steps 2.1.10 through 2.1.13. Complete the steps for all 8 wells: A1, A12, C4, C9, F4, F9, H1, H12.

2.1.14 After reading the last well (H12) press Enter and the 9700 system will begin the second reading (94ºC setpoint). The TNU Performance screen appears with the setpoint displayed at 94ºC the cover temp will increase and must be within 1ºC of 105ºC. It may take a few minutes for the system to stabilize at the setpoint temperature.

2.1.15 When the system has stabilized the TNU Performance screen with the "place probe in well A1" prompt appears. Repeat steps 2.1.9 to 2.1.13.

2.1.16 After the last reading for setpoint 94ºC is completed, the system 9700 evaluates the uniformity of the sample block temperature for the setpoint values that were entered and displays the results. A summary screen appears at the conclusion of the test.

Well	94ºC	37ºC		Well	94ºC	37ºC
A1	xx.x	xx.x		F4	xx.x	xx.x
A12	xx.x	xx.x		F9	xx.x	xx.x
C4	xx.x	xx.x		H1	xx.x	xx.x
C9	xx.x	xx.x		H12	xx.x	xx.x
Accept			More			Cancel
F1		F2	F3		F4	F5

2.1.17 Record the results on the Temperature Non-Uniformity Test Data Sheet (Appendix J), then select Accept (F1).

2.1.18 When the system completes the Temperature Non-uniformity test, the TNU Performance screen appears with either "Pass" or "Fail" after each setpoint.

2.1.18.1If the temperature of the sample block wells is uniform then the test has passed.

2.1.18.2 If the temperature variation of the sample block wells exceeds performance expectations the test has failed.

2.1.18.2.1 If the test fails, repeat the procedure to make sure the digital thermometer was not misread or that errors were not made entering data.

2.1.18.2.2 If the test fails again, contact Applied Biosystems Technical Support.

2.1.19 End the test by selecting Cancel (F5).

2.1.20 If all measurements are completed, remove the probe and the Temperature Verification Frame from the sample block and turn off the digital thermometer.

2.1.21 Clean the wells of the sample block using cotton swabs.

3 SYSTEM PERFORMANCE DIAGNOSTICS: RATE TEST AND CYCLE TEST

3.1 Testing the integrity of the cooling and heating system

Perform the procedure as described in the following steps:

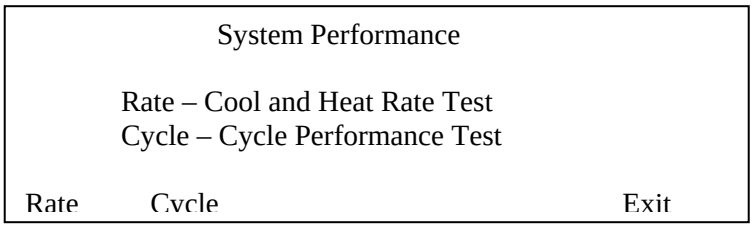
3.1.1 Before beginning the tests, an empty 96-well plate with full plate cover or caps must be in the sample block.

3.1.2 Turn on the GeneAmp PCR System 9700. The main menu appears:

3.1.2.1 Select Utilities (F4)
3.1.2.2 Select Diagnostics (F1)

3.1.2.3 Select System (F2)

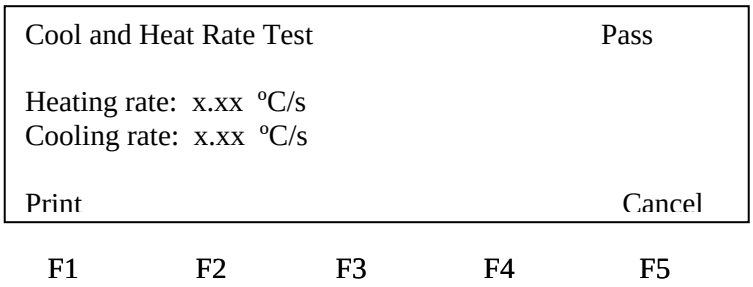
3.1.3 The System Performance Screen appears



3.1.4 Select Rate Test (F1) to verify that the Peltier units are operating correctly. The System Performance screen displays the following message: “WARNING!! Install the appropriate empty consumables into the sample block. Refer to System Performance Section of the Block User Manual.”

3.1.5 Select Cont. (F1) after the plate and cover has been installed and the Cool and Heat test screen appears.

3.1.6 The instrument then runs through a series of tests where the sample block ramps to and stabilize at 35ºC, 94ºC and 4ºC, which is displayed on the screen. At the conclusion of the test, the Cool and Heat screen appears displaying the results and whether the test results passed or failed. Record this information in the Cool and Heat Rate Test area on the worksheet (Appendix J). If the test fails contact Applied Biosystems Technical Support.



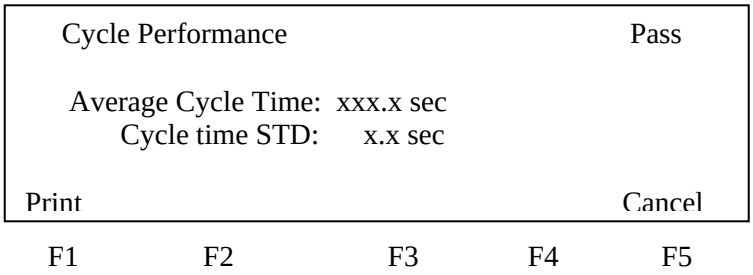
3.1.7 The following table lists the passing ranges for the Rate test:

	Heating Rate	**Cooling Rate**
Aluminum 96-well	1.5 to 2.5 ºC/second	1.5 to 2.5 ºC/second

3.1.8 Return to the System Performance screen by selecting Cancel (F5) and select Cycle Test (F2) to verify that the PCR cycling function operates properly. The System Performance screen displays the following message: "WARNING!! Install the appropriate empty consumables into the sample block. Refer to System Performance Section of the Block User Manual."

3.1.9 Select Cont. (F1) after the plate and cover has been installed.

3.1.10 The Cycle Test executes a standard PCR cycle reaction (6 cycles), measures, and reports the average cycle time, and the cycle to cycle variation. At the conclusion of the test, the Cycle Test screen will display the results and whether the test results passed or failed. Record this information in the Cycle Performance area on the worksheet (Appendix J). If the test fails contact Applied Biosystems Technical Support.



3.1.11 The following table lists the passing ranges for the Cycle Test.

Average Cycle Time	<= 160 sec
Cycle Time STD	< 5 sec

3.1.12 Select Cancel (F5) to exit the test.

♦END